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(54) Microspheres for controlled release and processes to prepare these microspheres

(57) The present invention relates to a process for the preparation of a controlled release system, comprising forming of an aqueous two-phase system from at least two water soluble polymers, which polymers are incompatible in solution, at least one of these polymers being crosslinkable, the crosslinkable polymer phase being emulsified in the other polymer phase; adding at least one releasable compound which is soluble in the crosslinkable polymer phase in the aqueous solution, allowing the releasable compound to diffuse in the crosslinkable polymer phase; crosslinking the crosslinkable polymer to a degree that the pores in the crosslinked structure are substantially smaller than the particles size of the releasable compound; and separating the crosslinked structures enclosing the releasable compound from the other phase. Further, the invention relates to microspheres, at least 80 wt.% thereof having a particle size of between 1 nanometer and 50 µm, which microspheres are comprised of a degradable, crosslinked polymer encapsulating at least one releasable compound, the pore size of the crosslinked polymer being smaller than the particle size of the releasable compound.

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Description

The present invention relates to microspheres which have a good controlled release behaviour, and to processes to prepare these microspheres.

The fast developments in the biotechnological field lead to a large number of pharmaceutically interesting products, esp. proteins, peptides and genes. Such products can suitably be used in the treatment of life-threatening diseases, e.g. cancer, and several types of viral, bacterial and parasital diseases.

Due to their nature, proteins and proteinaceous products, e.g. peptides, which group of products will be referred to as protein drugs herein-below, cannot efficiently be administered orally. They have to be brought in the system parenterally, i.e. by injection. The pharmacokinetical profile of these products is such that injection of the product per se requires a frequent administration. In other words, since protein drugs are chemically and physically unstable in the gastro intestinal tract and generally have a short active residence time in the human or animal body, multiple injections in a short time are required to attain a therapeutic effect. It will be evident that this is inconvenient for patients requiring these protein drugs.

For this reason, there is a need for delivery systems which have the capacity for sustained release. A number of options have been proposed in the art, and synthetic biodegradable, rather well-defined polymers are used to control the release of encapsulated drugs.

One of the options given in the prior art is the use of microspheres made of polymers. These microspheres or nanospheres are spherical particles, spherical capsules, nanocapsules or nanoparticles having a particle diameter between about 0.1 µm and about 100 µm. In this description and the claims, the reference to microspheres also encompasses microparticles, microcapsules, nanospheres, nanoparticles and nanocapsules. Widely used polymers to prepare these microspheres are poly lactic acid and copolymers of lactic acid with glycolic acid. The polymers should preferably be biodegradable to avoid removal of the polymer carrier after use.

The hitherto known preparation methods for drug containing controlled or sustained release systems generally involve the use of organic solvents. Organic solvents may lead to structural changes in protein structure, esp. in the secondary and tertiary structure. Such changes may lead to a denaturation of the protein drug. Since these structural changes normally lead to a loss in pharmacological activity and the occurence of undesired side-effects, such changes are undesirable, as will be apparent. Moreover, the use of organic solvents is not desirable from an environmental point of view, either.

Further, it is hardly possible to avoid that traces of organic solvents will remain in or on the microspheres produced. Especially, when toxic solvents are used, such as the widely applied solvents chloroform and dichloromethane, this is a problem.

Another problem is that it is difficult to encapsulate proteins in polymeric matrices in a reproducible way. It is of the utmost importance that predictable and reproducible amounts of proteins or other encapsulated products to be used as drugs are released.

Hydrogels have been used in the preparation of delivery systems for protein drugs. One of these systems comprises crosslinked dextrans obtained by radical polymerization of glycidyl methacrylate derivatized dextran (dex-GMA). In this respect, reference is made to Van Dijk-Wolthuis et al. in Macromolecules 28, (1995), 6317-6322 and to De Smedt et al. in Macromolecules 28, (1995) 5082-5088.

It appeared that the release of the proteins from these hydrogels depends on and can be controlled by the degree of crosslinking and the initial water content of the gel (Hennink et al., J. of. Contr. Rel. 39 (1996), 47-57).

Drugs are usually loaded into hydrogels or microspheres derived hereof either by equilibration in a drug-containing solution followed by drying (see e.g. Kim et al. in Pharm. Res. 9(3) (1992) 283-290) or by incorporation of the drug during the preparation of carrier (see e.g. Heller et al. in Biomaterials 4 (1983) 262-266). Both techniques have a number of disadvantages other than those arising from any organic solvents used.

Loading by equilibration normally leads to a rather low drug content in the delivery system. This is especially the case, when the drug is a macromolecular compound. Unless the pore size of the hydrogel or the microsphere is rather large, the macromolecules will only adsorb onto the outer surface, which may lead to a burst release. Further, the solvent phase containing the drug, which is contacted with the delivery system to load the delivery system has to be removed from the hydrogel or the microspheres. This can produce the migration of the drug to the surface of the delivery system, and hence to a non-homogeneous drug distribution. This tends to result in a significant burst release of the drug, as well, which generally is not desired.

The drugs are released from these hydrogels or polymeric microspheres during biodegradation of the polymeric material and/or by diffusion.

A suitable loading process for incorporating macromolecular drugs is aimed at.

In an article in Proceed. Intern. Symp. Control. Rel. Bioact. Mater., 22 (1995), 145-146, Gehrke et al. have described a technique wherein loading levels higher than obtainable by solution sorption, hence higher than about 0.1 wt.%, can be achieved in purified, pre-formed hydrogels. The loading technique is based on the fact that certain poly-

mer mixtures split into separate phases when dissolved in water. Proteins dissolved in such a system distribute unevenly between the phases. This principle also holds when one of the polymer phases is a crosslinked gel.

In particular, Gehrke et al. describe a crosslinked dextran gel/poly(ethylene glycol) system, and a crosslinked hydroxypropylcellulose gel/poly(vinyl alcohol) system. Proteins present in an aqueous solution containing beads of the gel are, after the addition of the non-crosslinked second polymer, adsorbed on the beads and partly absorbed through meshes or pores in the bead surfaces.

A disadvantage of this technique is that the proteinaceous material is to a major extent only adsorbed to the beads, which means that if the phase containing the second polymer is replaced by another aqueous system a fast removal of the proteins from the beads is observed. Only when large amounts of pores having a diameter larger than the size of the proteinaceous material to be loaded are present in the bead surfaces, some absorption may occur. This adsorption and limited absorption behaviour has an undesirable effect on the release of the proteinaceous material from the beads.

To additionally illustrate the undesired release behaviour, it is noted that the profiles shown in the article are - from a pharmacological point of view - entirely unsuitable to be used in controlled release systems. Moreover, the gel beads are too large (cylinders with a diameter of 1.5 mm) to be suitably used for administration in the human or animal body.

The principle of affinity partitioning in PEG-containing two-phase systems is also known from Göte Johansson, Affinity Partitioning in PEG-containing Two-phase Systems In: Topics in Applied Chemistry, Poly(ethylene glycol) chemistry, Biotechnological and Biomedical Applications, Ed. J.M. Harris, Plenum Press (1992). In this article, a two-phase system is described, which is created when an aqueous solution of dextran and polyethylene glycol (PEG) are mixed. A PEG enriched and a dextran enriched phase are formed. Proteins are partitioned unequally in such systems. These known systems are used in the purification of proteins.

The present invention is aimed at providing a new injectable, patient friendly delivery system for protein drugs, which system is safe and biodegradable, and which system possesses well controllable delivery kinetics. The period wherein drug delivery should be guaranteed depends on the protein drug used, and varies between a number of days upto more than one year. In addition, high degrees of loading in the delivery system can be obtained. Moreover, the system of the present invention can be produced without needing the use of organic solvents.

The problems mentioned above are solved by a specific preparation method of microspheres wherein water is used as the solvent. The use of water as sole solvent system is advantageous from an environmental point of view, because of toxicological considerations and, especially, because of reasons of protein stability.

In a first aspect, the present invention relates to a process for the preparation of a controlled release system, comprising forming of an aqueous two-phase system from at least two water soluble polymers, which polymers are incompatible in solution, at least one of these polymers being crosslinkable, the crosslinkable polymer phase being emulsified in the other polymer phase, and crosslinking of the crosslinkable polymer.

The process of the invention further normally comprises the steps of adding at least one releasable compound which is soluble in the crosslinkable polymer phase in the aqueous solution, and allowing the releasable compound to diffuse in the crosslinkable polymer phase, before the crosslinkable polymer is crosslinked.

By emulsifying an aqueous crosslinkable polymer in a continuous phase comprised of water and a polymer which is not compatible with the crosslinkable polymer, and crosslinking the discontinuous phase, the particle size of the crosslinked polymer particles can be adjusted, while the particle size distribution can be narrow.

In a preferred embodiment, the crosslinking is carried out to such a degree that the pores (meshes) in the crosslinked structure are substantially smaller than the size of the releasable compound. In this embodiment, the crosslinked structure must be degradable in the human or animal body, so that the encapsulated releasable compound can leave the crosslinked matrix.

The degradability of the crosslinked structure can be regulated in a number of ways. As a first example, it is noted that bonds can be incorporated, which are hydrolysable under physiological conditions. In this respect, reference can be made to the European patent application 96201821.4 of the group of the present inventors. This patent application teaches hydrogels comprising hydrolytically labile spacers between different polymer chains. The hydrolytically labile spacers described therein can be suitably used in the present invention and are incorporated by reference.

Another example to control the degradability is the coencapsulation of an enzyme or chemical substance capable of breaking bonds in the crosslinked polymer. In a preferred embodiment of the process of the present invention the crosslinkable polymer is a dextran polymer. In this embodiment a dextranase can be added to the aqueous two-phase system before the crosslinking step or added afterwards.

The dextran polymer can suitably be used in the process of the present invention together with a polyethylene glycol or Pluronic®, which is a preferred polymer to be used in the inventive process.

The product aimed at by the process of the present invention can be separated from the other polymer phase.

In a further aspect, the present invention is directed to microspheres, at least 80 wt.% thereof having a particle size of between 100 nanometer and 100 μm, which microspheres are comprised of a degradable, crosslinked polymer encapsulating at least one releasable compound, the pore size of the crosslinked polymer being equal or preferably smaller than the particle size of the releasable compound. These microspheres are obtainable by using the process of

the invention, and are free from organic solvents. Dependent on the application of the microspheres, the size can e.g. be adjusted between 1 and 50 µm, preferably between 2 µm and 25 µm, such as between 5 and 15 µm.

When the pore sizes or meshes of the crosslinked polymer are equal or smaller than the hydrodynamic diameter size of the releasable component, the releasable component is essentially released when the polymer is degraded. If on the other hand, the pore sizes or meshes of the crosslinked polymer are larger than the size of the releasable component, the releasable component is at least partially released by diffusion. The pore size of the crosslinked product obtained by the process of the present invention in this way provides a perfect tool to control the release. Further, the efficiency of the incorporation of a releasable compound in such a polymer structure is very high, while the degree of loading can be adjusted up to the saturation concentration of the compound to be released.

The polymers used can be chosen dependent on the nature of the compound to be released. It is preferred that the compound to be released will have a clear preference for the crosslinkable polymer phase. In that case, the highest possible degree of loading, theoretically up to the saturation concentration, in the microspheres to be made, can be obtained.

It is not critical which crosslinkable polymer is used. However, if the controlled release system comprising the polymer in crosslinked form is intended to be brought into a human or animal body, the polymer should be pharmaceutically acceptable and preferably should be degradable. Suitable crosslinkable water soluble polymers are dextrans and derivatized dextrans, starches and starch derivatives, cellulose derivatives such as hydroxyethyl and hydroxypropyl cellulose, polyvinylpyrrolidone, proteins and derivatized proteins, and so on. The molecular weigth of the crosslinkable polymers used normally lies between 1,000 and 1,000,000 Da. It is noted that with a higher molecular weight of the polymer, a better phase separation is generally obtained in the aqueous solution used in the process of the invention.

The person skilled in the art will have the knowledge to choose the crosslinkable polymer and the crosslinking conditions required for the emulsion prepared. For instance, dextans can be crosslinked with methylacrylate or methacrylate groups. Another example is a system comprising PVP as the external phase and dextran as the emulsified phase, wherein the dextran is crosslinked through the presence of isocyanates.

Further, reference is made to crosslinking using radiation. Dex-GMA can e.g. be polymerized using small dosages of γ-radiation, such as less than 0.1 Mrad. An advantage of this embodiment is that in one step sterile microparticles can be obtained. Further, crosslinking by UV radiation and physical crosslinking using e.g. hydrophobic tails coupled to a polymer are possible techniques.

In a preferred embodiment, the crosslinkable polymer is a temperature sensitive polymer such as poly-N-isopropylacrylamide, which polymer can e.g. be present as a graft on another polymer such as a dextran. Hydrogels of these polymers show increasing swelling behaviour at degreasing temperatures. This makes it possible that releasable material can easily penetrate in the hydrogel. By subsequently raising the temperature, e.g. to a value of 37°C, the meshes in the hydrogel shrink, thereby capturing the releasable compound.

The release of the releasable compound depends on a number of variables, which can be used to tailor the delivery as desired. One of these variables is the size of the microspheres. The size can be adjusted by carefully modifying the process circumstances and formulation parameters in the emulsifying step. For instance, the water content, the presence of hydrophobic groups on any one of the polymers used, the viscosity of the continuous and discontinuous phase, and the electrical charge on the at least two polymers used are examples of tools to adjust the size of the microspheres or microparticles to be produced. In addition, emulsifiers can be added. Suitable emulsifiers are copolymers, preferably block-copolymers, of units of the two incompatible polymers, e.g. a block-copolymer of PEG and dextran, used to create the two-phase system.

To further guarantee a controlled release, the crosslinked polymer should preferably be degradable.

The polymer which is present in the aqueous continuous phase can be any polymer which is incompatible with the crosslinkable polymer. Although this polymer may also be crosslinkable, but of course not under the reaction conditions used for the crosslinking of the discontinuous polymer phase, this is not preferred. Examples of suitable polymers incompatible with the polymer to be crosslinked are poly(ethylene glycol) (PEG) and poly(vinyl alcohol) (PVA) (in combination with e.g. dextrans and dextran derivatives, starches and starch derivatives, PVP, and water soluble cellulose derivatives).

As indicated herein-above, the releasable compound can be a protein drug. However, it is also possible to encapsulate pharmacon containing nanoparticles or microparticles, e.g. liposomes and iscoms. The encapsulation of this type of particles has the advantage of preventing the occurence of a too fast release of the encapsulated compound, or, said in other words, burst-effects can be avoided in a more secure way.

The partition of the compound to be released is firstly determined by the nature of the polymers present in the aqueous two-phase system. This partition can be influenced, e.g. by adding salt to the aqueous system, or by adjusting the pH.

If the releasable compounds, such as proteins, are present during the crosslinking step, care should be taken that the integrity of the releasable compounds is secured. It should for instance be avoided that proteinaceous material is oxidized by initiator systems etc. In this light, it is noted that adverse effects can be avoided or minimalized by minimal-

izing the amount of initiator, reducing the polymerization time or adding suitable antioxidantia, such as α-tocopherol.

The separation of the crosslinked structures enclosing the releasable compound from the other phase can be carried out in any conventional way. Preferably, the separation is effected by filtration or centrifugation. The crosslinked structures can subsequently be washed with water and dried. The drying step determines that a pharmaceutically acceptable product can be obtained, having a maintenance term of more than 2 years. A very preferred drying method is spray-drying, although the drying can also be suitably carried out using lyophilization.

In a first step of the process of the present invention an aqueous two-phase system is formed. This two-phase system comprises water, and at least two water soluble polymers, which polymers are incompatible in solution. Preferably a compound to be released is also present, although it is possible to add the compound to be released after the crosslinking step, e.g. when using heat sensitive polymers that increasingly with decreasing temperature swell. At least one of the polymers present in the aqueous phase is crosslinkable, and the crosslinkable polymer phase is emulsified in the other aqueous polymer phase.

The process of the present invention will now be further illustrated by the preparation of dextran microspheres using the water-in-water emulsion technique, and by the following, non-limiting examples.

Example 1

Polyethylene glycols (PEG) with varying molecular weights were obtained from Merck-Schuchardt, Germany. Glycidyl methacrylate derivatized dextrans (dex-GMA) with varying DS (degree of substitution; the number of methacrylate groups per 100 glucopyranose residues) were synthesized by a coupling reaction of dextran T40 and glycidyl methacrylate in DMSO (dimethylsulfoxide) using DMAP (N,N-dimethylaminopyridine) as a catalyst, essentially as described by Van Dijk-Wolthuis et al. in Macromolecules 28, (1995) 6317-6322.

Dex-PEG was synthesized as follows. mPEG (monomethoxypolyethylene glycol, M 5000 g/mmol, 5 g; corresponding with 1 mmol hydroxyl groups) and CDI (carbonyldiimidazole, 162 mg, 1 mmol) were dissolved in 100 ml of anhydrous tetrahydrofuran. The solution was stirred overnight at room temperature, followed by the evaporation of the solvent under reduced pressure. Next, the CI (carbonylimidazole) activated mPEG was added to a solution of dextran T40 (1.7 g) and DMAP (0.35 g) in 50 ml DMSO. This solution was stirred for one week at room temperature. After neutralization of DMAP with HCl, the solution was extensively dialyzed against water and subsequently freeze dried. The product was characterized by gel permeation chromatography and NMR. The degree of substitution amounted to 4. Dex-lactate-HEMA (DS 3) was synthesized as described in the copending European patent application No. 96201821.4.

Polyethylene glycol (PEG, varying molecular weight) was dissolved in 0.22 M KCl to a concentration of 12-40% (w/w). Dex-GMA was dissolved in 0.22 M KCl to a concentration of 10-40% (w/w). Both solutions were flushed with nitrogen for 10 minutes. Next, 4.75 ml of the PEG solution and 0.25 ml of the dex-GMA solution were mixed and vortexed (Winn Vortex-Genie, maximum speed) for 1 minute resulting in a water-in-water emulsion with dextran as the inner phase and PEG as the outer phase. After 10 minutes TEMED ((N,N,N',N'-tetramethylethylenediamine, 100 µl, 20% (v/v) in 0.22 M KCl, pH adjusted with concentrated HCl to 7.2) and KPS (potassium peroxydisulfate, 180 µl, 50 mg/ml in water) were added. The emulsion was incubated for 30 minutes at 37°C to polymerize the dex-GMA. The microspheres were washed twice with water and freeze dried.

It was demonstrated using *in vitro* cell cultures that cytotoxicity of dex-GMA is low in similar to the cytotoxicity of dextran, which compound has been used for years as plasma replacing agent in human beings.

The particle size (number weight diameter ($=\Sigma nd/\Sigma n$) and volume weight diameter ($=\Sigma nd^4/\Sigma nd^3$)), I.C. Edmundson, Particle size analysis, H.S. Bean, A.H. Beckett and J.E. Carles (eds) in: Advances in Pharmaceutical Sciences vol.2, Academic Press, London 1967, 95-174) and particle size distribution were determined by a laser light blocking technique (Accusizer™, model 770, Particle Sizing Systems, Santa Barbara, CA, USA). The shape and surface characteristics (porosity) of the microspheres were established by scanning electron microscopy (SEM) analysis.

Figure 1 gives a representative example of particle size distribution of a dextran microsphere batch prepared via the water-in-water emulsion technique as determined using the Accusizer. SEM analysis showed that the particles are perfectly spherical and non-porous (Figure 2).

Figure 3 shows the volume weight average diameter of dextran micropsheres as a function of the degree of GMA substitution and the molecular weight of PEG. The concentration of the PEG solution was 24% (w/w); the concentration of the dex-GMA concentration was 20%. It is shown that the particle size increases with decreasing molecular weight of PEG. At a fixed molecular weight of PEG, the particle size slightly decreases with increasing DS.

Figure 4 shows the volume weight average diameter of dextran micropsheres as a function of the degree of GMA substitution and concentration of the aqueous dex-GMA concentration. The mean diameter decreases with decreasing dex-GMA concentration.

Figure 5 shows the effect of the concentration and molecular weight of PEG on the volume weight average diameter of the dextran microspheres. For this evaluation, dex-GMA with a DS of 8 in 0.22 M KCl (20%, w/w) was used. It

appears that for a given PEG, the largest particles were obtained at a PEG concentration around 24%.

Example 2

The water-in-water emulsion can also be prepared using a mechanical stirrer instead of the vortex used in Example 1. A solution of dex-GMA (DS 4,7,13 or 30; 20% w/w) in 0.22 M KCl was added to a solution of PEG (varying molecular weight; 24%) in 0.22 M KCl, and mechanically stirred (around 600-1000 rpm, depending on the viscosity of the PEG phase) for 5 minutes under a nitrogen stream. Next, TEMED ((N,N,N',N'-tetramethylethylenediamine, 1.0 ml, 20% (v/v) in 0.22 M KCl, pH adjusted with concentrated HCl to 7.2) and KPS (potassium peroxydisulfate, 1.8 ml, 50 mg/ml in water) were added and the mixture was incubated for 30 minutes at 37°C to polymerize the dex-GMA. The particle size is slightly greater using a mechanical stirrer instead of a vortex (Figure 5).

Example 3

Microspheres were prepared from the following formulations using the protocol as given in Example 1 and using the following stock solutions:

Stock solutions (% in w/w) in 0.22 M KCl:

A. PEG 10.000, 24%
B. PEG 20.000, 24%
C. Dex-GMA (DS 13) 20%
D. Dex-lactHEMA (DS 3) 20%
E. Dex-lactHEMA (DS 3) 10%
F. Dex-PEG 20%

Table 1 summarizes the results:

PEG	dex	emulsifier	number weight diameter (µm)	volume weight diameter (µm)
4.50 ml A	0.25 ml C	0.25 ml F	3.3	7.2
4.75 ml A	0.25 ml C	no	4.4	11.5
4.75 ml B	0.25 ml D	no	6.3	17.0
4.75 ml B	0.25 ml E	no	5.3	16.0

As can be seen, a suitable emulsifier (block-copolymer of dextran and PEG) gives smaller particles with a smaller dispersity (= weight mean diameter/number mean diameter).

Example 4

The release of a model protein from non-degrading dextran microspheres and degradable microspheres was evaluated. The microspheres were rendered degradable by the incorporation of dextranase in dex-GMA microspheres.

Dex-GMA (DS 8) was dissolved in 10 mM phosphate buffer pH 8.0. To 2 ml of this solution a fixed amount of IgG (immunoglobuline G, 25.6 mg) and a variable amount of dextranase (Sigma D1508; 0, 0.1 and 1 U (1 U releases 1 µmol reducing oligosaccharides per minute at 37°C and pH 6.0)) dextranase were added. This solution was emulsified in an aqueous solution of PEG (M 10.000, concentration 24% (w/w)) in 0.22 M KCl. Thereafter, TEMED (N,N,N',N'-tetramethylethylenediamine, 100 µl, 20% (v/v) in 0.22 M KCl, pH adjusted with concentrated HCl to 7.2) and KPS (potassium peroxydisulfate, 180 µl, 50 mg/ml in water) were added. The microspheres were washed with water and dried under a nitrogen flow.

An accurately weighed amount (0.3-0.5 g) of microspheres was suspended in 10 ml phosphate buffer pH 5.5 and the amount of protein released in the buffer was determined using the biorad protein assay (M. Bradford. Anal. Biochem. 72 (1976) 248-254). Figure 7 shows the release profiles. From this figure it is clear that the release of IgG from dextran microspheres can be modulated by dextranase.

Claims

1. Process for the preparation of a controlled release system, comprising forming of an aqueous two-phase system from at least two water soluble polymers, which polymers are incompatible in solution, at least one of these polymers being crosslinkable, the crosslinkable polymer phase being emulsified in the other polymer phase; and crosslinking of the crosslinkable polymer.

2. The process of claim 1, comprising the steps of adding at least one releasable compound which is soluble in the crosslinkable polymer phase in the aqueous solution, and allowing the releasable compound to dissolve or diffuse in the crosslinkable polymer phase, before the crosslinkable polymer is crosslinked.

3. The process of claim 2, wherein the crosslinking is carried out to such a degree that the pores in the crosslinked structure are substantially smaller than the size of the releasable compound.

4. The process of any one of the preceding claims wherein before or after the crosslinking step an enzyme capable of degrading the matrix formed is added to the aqueous two-phase system.

5. The process of any one of the preceding claims, wherein the crosslinkable polymer is a dextran polymer.

6. The process of claim 5, wherein before or after the crosslinking step dextranase is added to the aqueous two-phase system.

7. The process of any one of the preceding claims, wherein one of the polymers is polyethylene glycol.

8. The process of any one of the preceding claims, wherein the crosslinked structures are separated from the other polymer phase.

9. Microspheres, at least 80 wt% thereof having a particle size of between 100 nanometer and 100 µm, preferably between 5 and 15 µm, which microspheres are comprised of a degradable, crosslinked polymer encapsulating at least one releasable compound, the pore size of the crosslinked polymer being smaller than the particle size of the releasable compound.

10. Microspheres according to claim 9, being free from organic solvent.

Figure 1

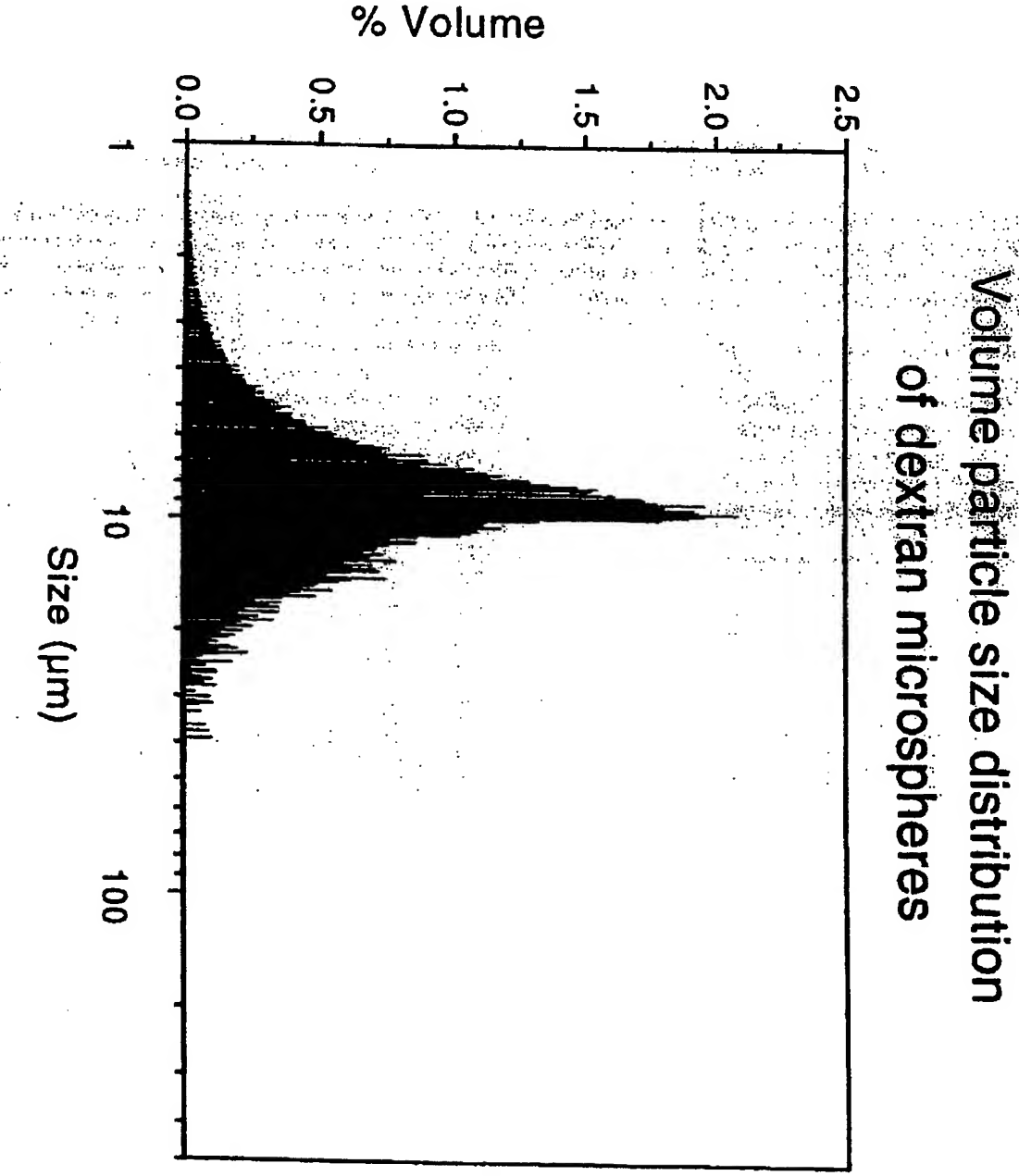


Figure 2

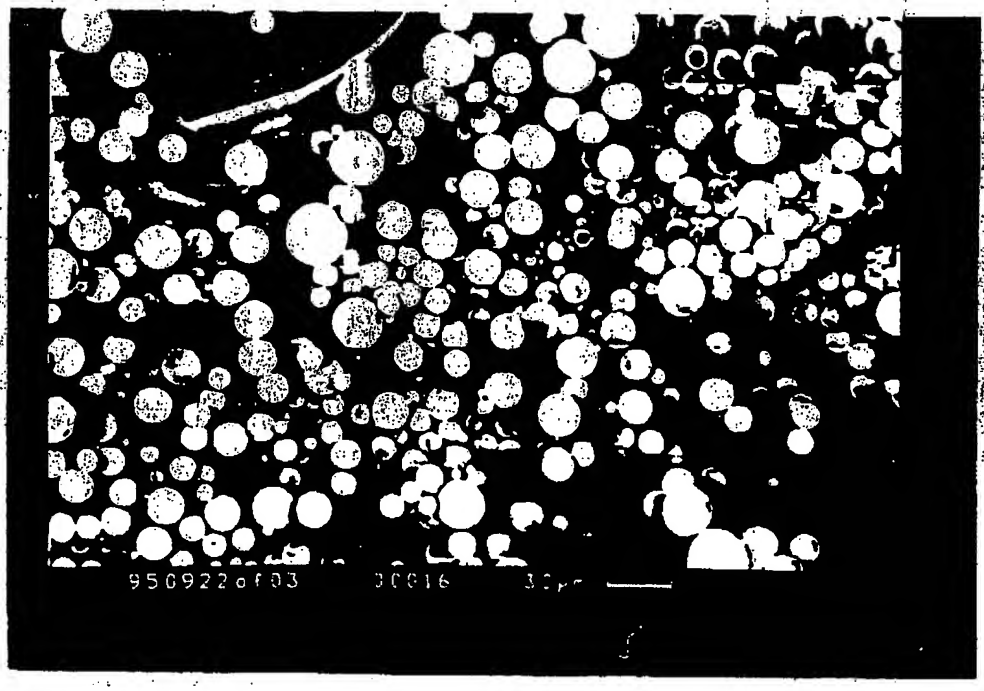


Figure 3

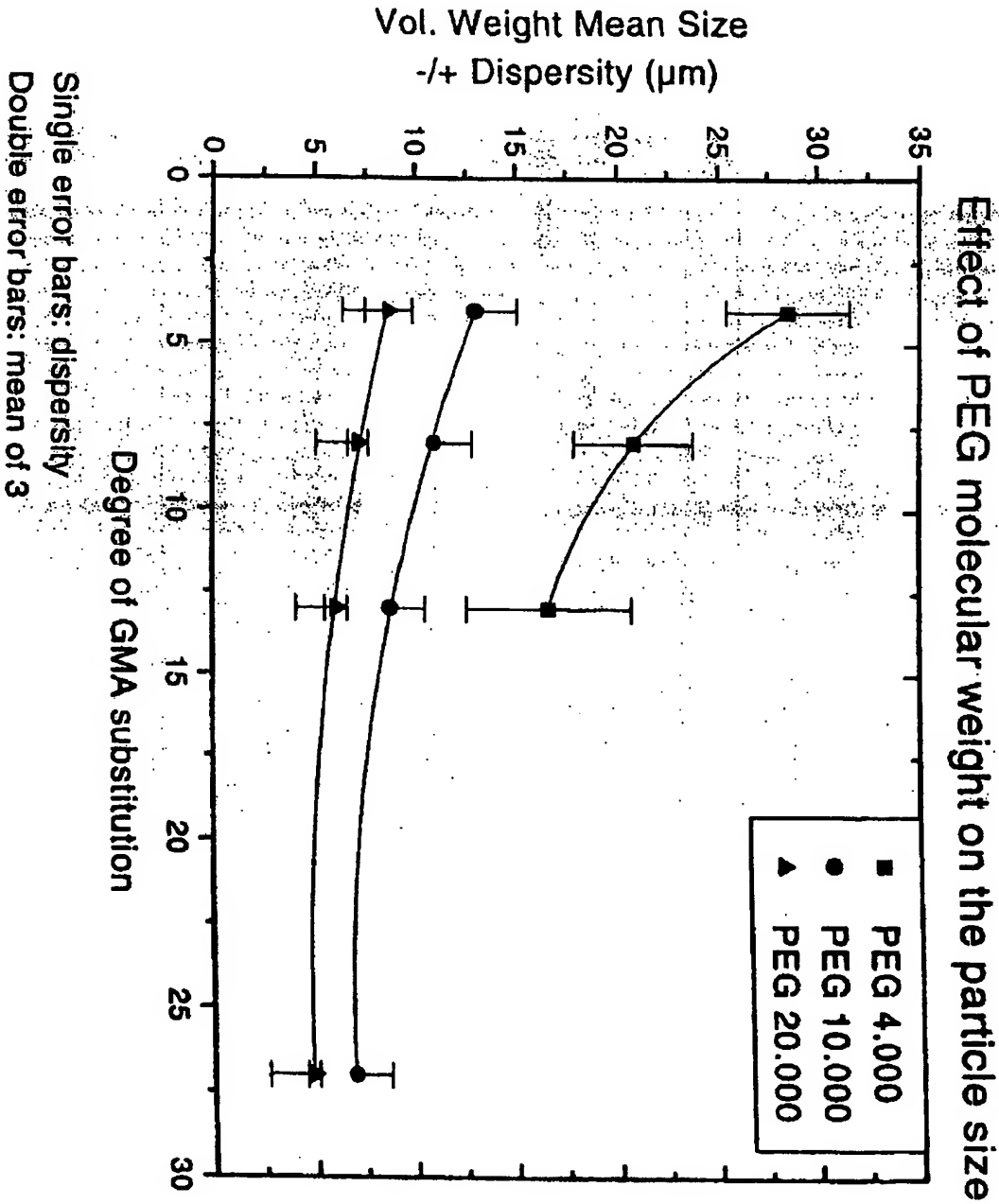


Figure 4

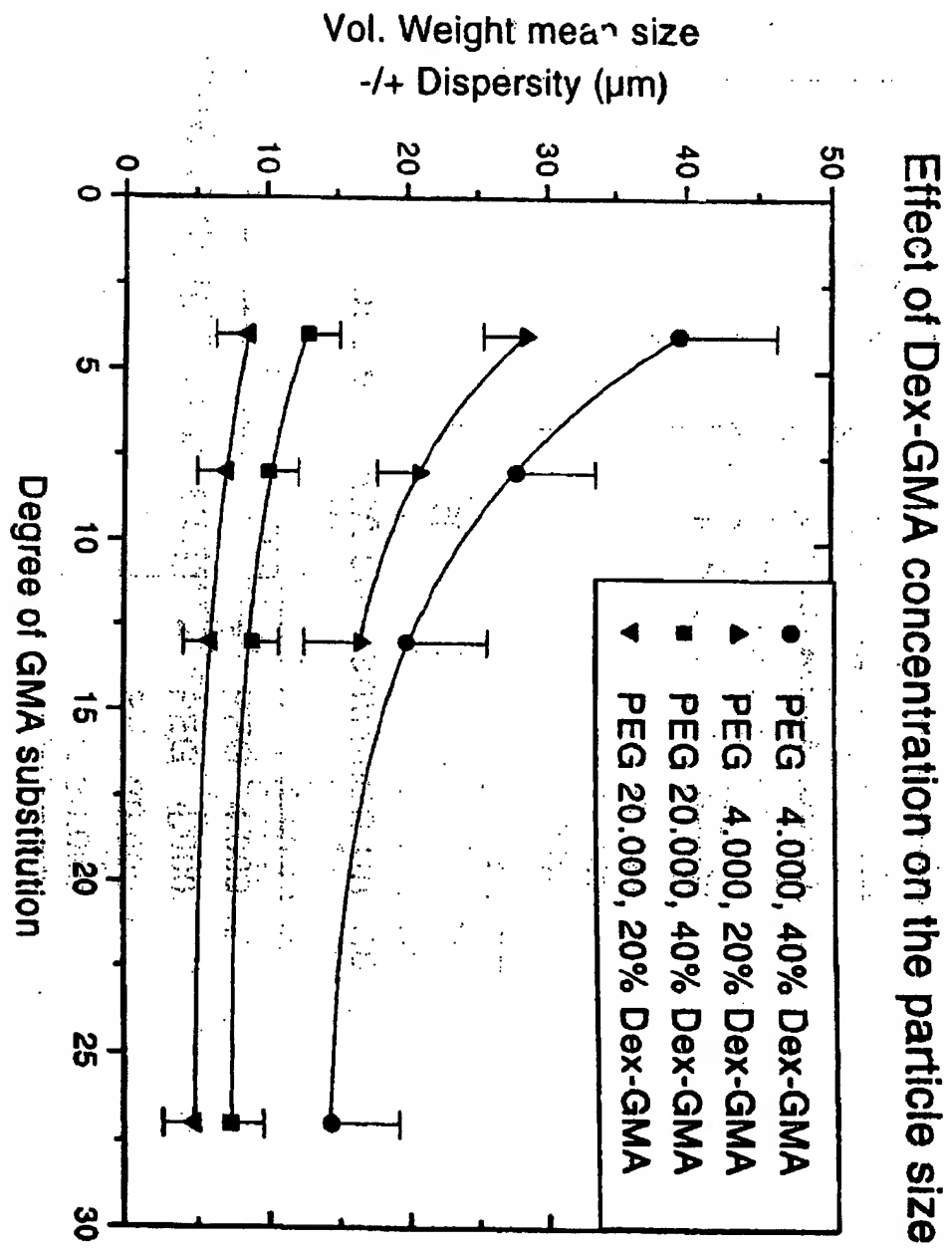
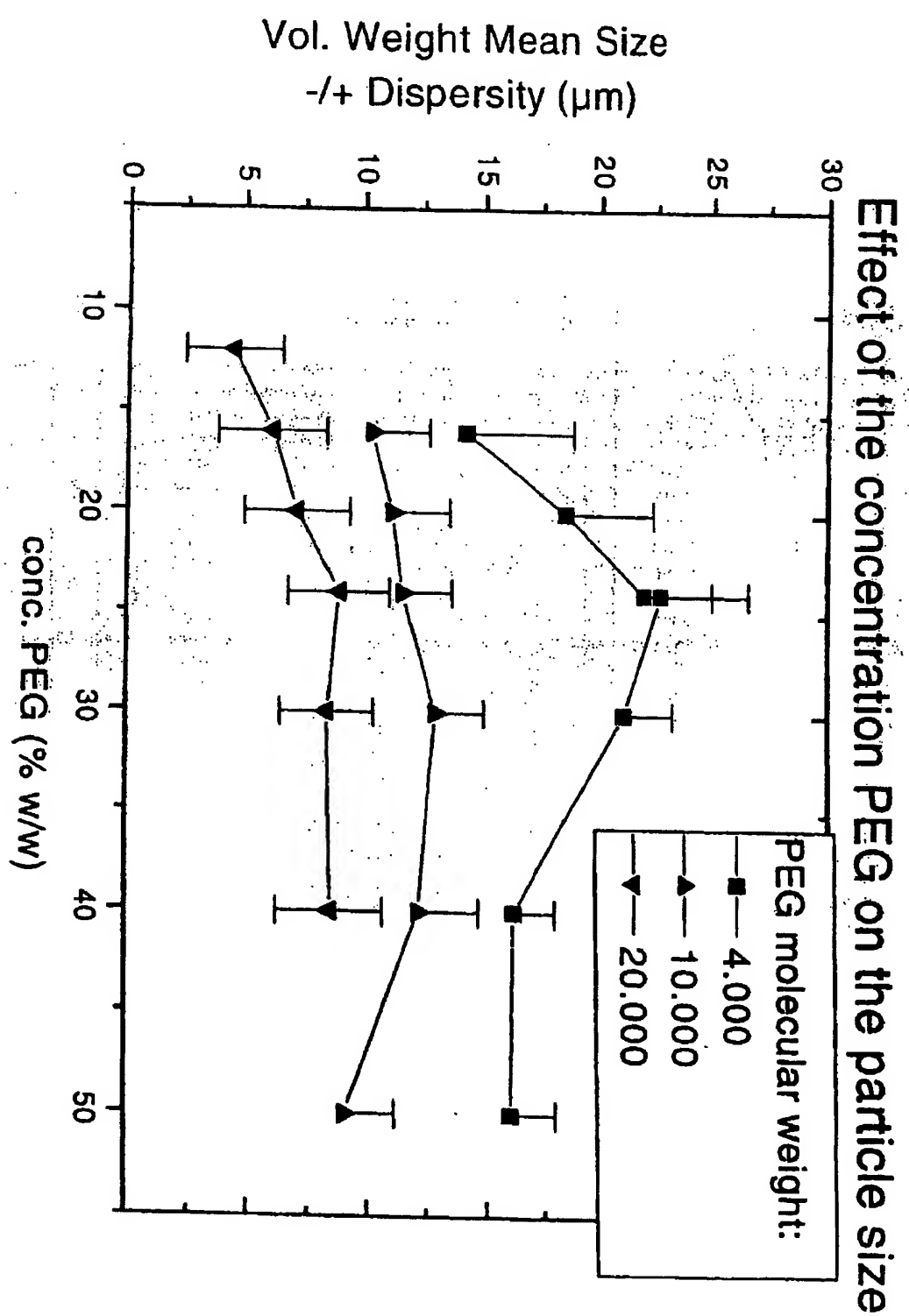


Figure 5


Effect of the concentration PEG on the particle size
Vol. Weight Mean Size
-/+ Dispersity (µm)
conc. PEG (% w/w)
PEG molecular weight:
4.000
10.000
20.000
0
5
10
15
20
25
30
10
20
30
40
50

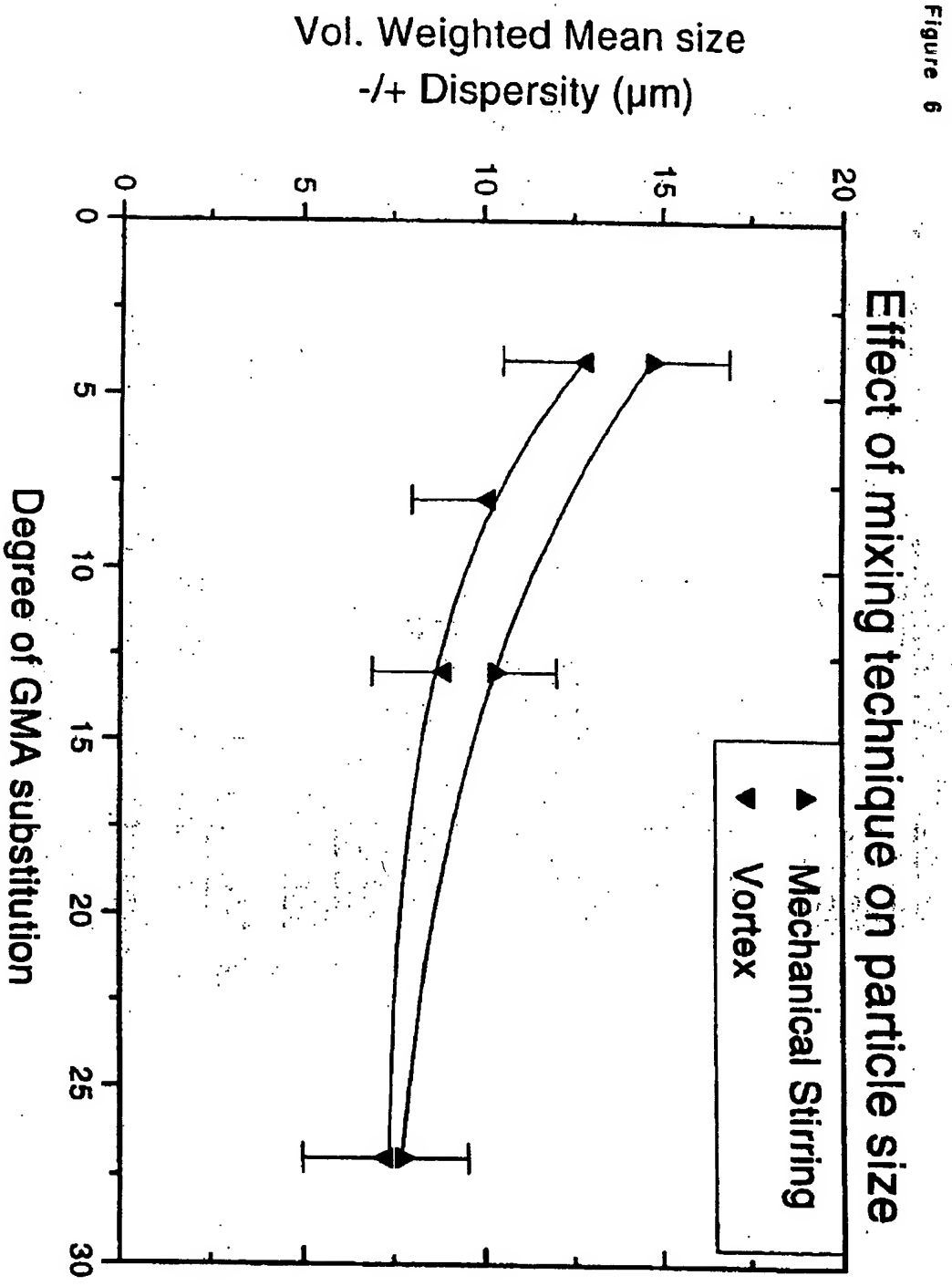
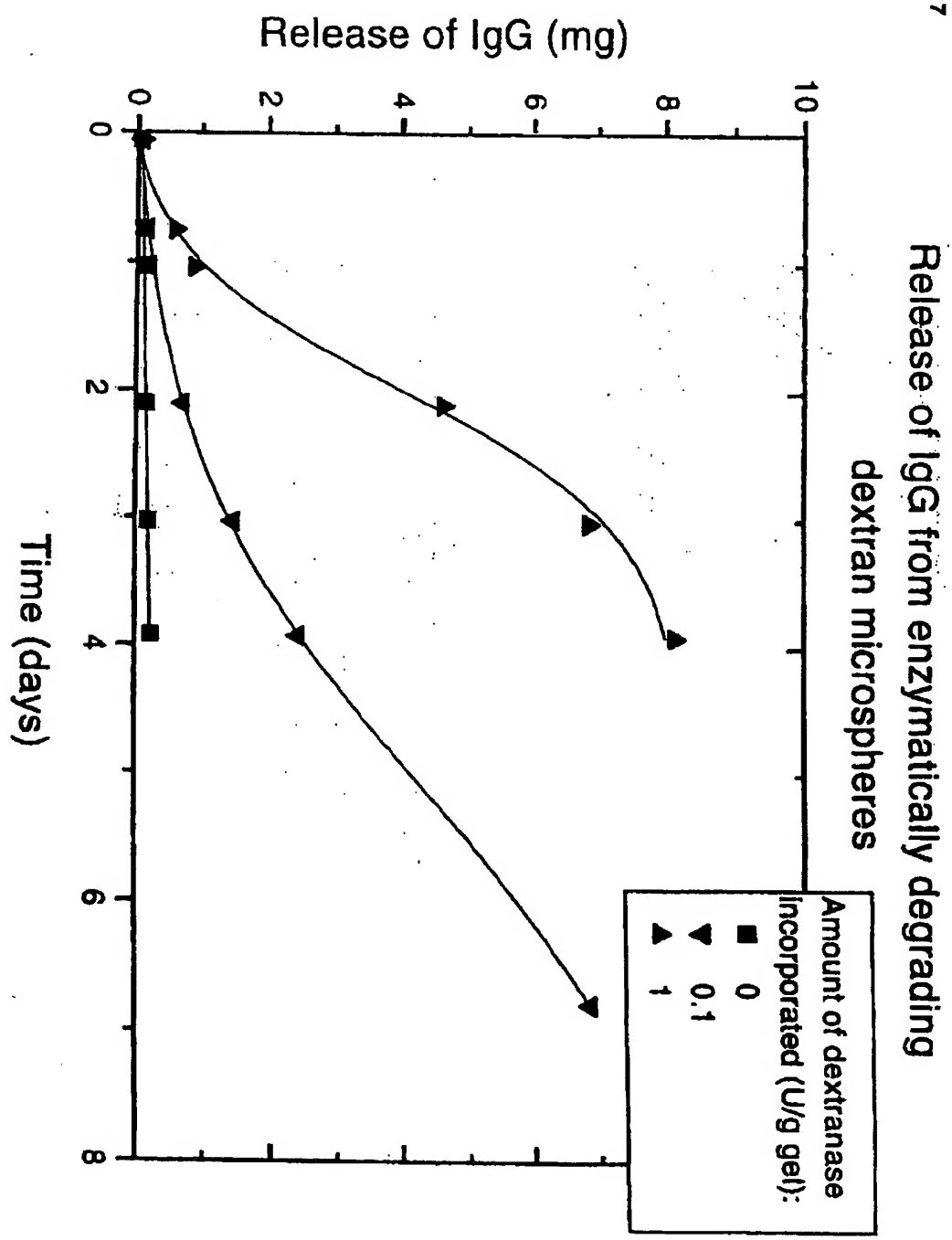


Figure 6

Figure 7



European Patent Office

EUROPEAN SEARCH REPORT

Application Number
EP 96 20 3234

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
X Y	EP 0 213 303 A (MAGNUS ET AL.) * column 1, line 1 - line 7 * * column 7, line 21 - line 44 * * column 10, line 21 - line 28 * * column 13; example 6 * * claim 10 * ---	1-3,5,7-10 4,6	A61K9/16
Y	WO 95 34328 A (ROYER) * page 6, line 18 - line 27 * * page 14, line 1 - line 8 * * page 15, line 24 - line 25 * * page 22; example 6; table 3 * ---	4,6	
Y	WO 96 02239 A (UNIVERSITY OF CINCINNATI) * the whole document * ---	1-3,5,7,8	
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TECHNICAL FIELDS SEARCHED (Int.Cl.6)
A61K

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	28 April 1997	Benz, K

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EUROPEAN SEARCH REPORT

Application Number
EP 96 20 3234

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
T	JOURNAL OF CONTROLLED RELEASE, vol. 39, no. 3, 1 December 1996, AMSTERDAM (NL), pages 237-248, XP000624385 K. MORIYAMA ET AL.: "regulated insulin release from biodegradable dextran hydrogels containing poly(ethylene glycol)" * the whole document *	1-10	

TECHNICAL FIELDS SEARCHED (Int.Cl.6)

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